The Remarks

Applicants thank the examiner for indicating that claim 4 would be allowable if written in independent form including all limitations of the base claim and any intervening claims. Claims 4 and 24 have been amended. Claims 1-3 and 5-23 have been canceled. No new matter has been added.

Claim 24 was rejected under 35 U.S.C. § 103(a) as being unpatentable specifically over the combination of U.S. Patent No. 5,955,349 ("Raymond") and EP 0195691 ("Thim"), in further view of U.S. Pat. Nos. 5,324,639 ("Brierly") and 6,337,193 ("Tully").

The process used to obtain the strain of the invention is not anticipated by the cited documents. In the present application, the method for obtaining the strain of *P. pastoris* of the invention, which is a high-yield producer of the precursor of human insulin, comprises transforming *P. pastoris* cells with vector pPIC9-Ib containing a single copy of the gene of interest (Examples 4 and 7). Transformation of the *P. pastoris* cells produced multiple HIS^+ recombinant clones, of which some were Mut^+, while others were Mut^s. As stated on page 37 of the description of the present application, the best yielding clone, deposited under ATCC No. PTA-2260, was a Mut^s clone containing a copy of the first DNA construction and 13 copies of the second DNA construction. In addition, it should be remarked that the first transformation was carried out with the pPIC9-Ib vector, designed to be incorporated into the yeast cells genome through a double recombination or replacement.

In the method of the invention, once the HIS^+ Mut^s clones and the HIS^+ Mut^+ clones had been selected and characterized with one or several copies of the gene of interest integrated into the AOXI gene or in the HIS gene, they were retransformed (second transformation event) into

said clones, with a second construction comprised in the pPICZαA-Ib vector, which contained from 1 to 8 copies of the gene of interest. Said vector was especially designed to be incorporated into the yeast genome by means of a simple insertion, thus obtaining the strain of the invention, which produces large amounts of the human insulin precursor.

Therefore, it is not obvious to those skilled in the art of genetic engineering that retransforming a strain with a single copy of the gene of interest by replacing the AOXI gene by a vector containing a single copy of the second DNA construction results in a strain that produces 400 mg/liter of the insulin (see Example 13). Those skilled in the art know that a high yield of the protein of interest is not directly correlated to the number of copies of the DNA sequence encoding it, since it often happens that great number of codifying sequences reduces the yield of protein.

Surprisingly, the highest yielding clone of *P. pastoris* was the one containing a single copy of the gene of interest after a first transformation event, which provided a Mut^S clone, which was subsequently retransformed with a vector containing only one copy of the gene of interest, and which was integrated into the cell genome through a simple insertion. Accordingly, Applicants respectfully request that the rejection to claim 24 be withdrawn.

CONCLUSION

Claims 4 and 24 are pending in this application and are in condition for allowance. Examination and allowance of the claims is respectfully requested. If the Examiner has any questions or concerns, the Examiner is invited to telephone the undersigned at (415) 954-0230.

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Respectfully submitted,

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